GLP-2 has differential effects on small intestine growth and function in fetal and neonatal pigs

Y. M. PETERSEN,1 D. G. BURRIN,2 AND P. T. SANGILD1

1Division of Animal Nutrition, Royal Veterinary and Agricultural University, Copenhagen, Denmark; and 2US Department of Agriculture/Agricultural Research Service Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030

Received 8 February 2001; accepted in final form 14 August 2001

The pig small intestine undergoes tremendous growth and functional changes in the immediate pre- and postnatal periods. In the final 20% of gestation, the mucosal mass (relative to body weight) increases by ~150% and is associated with significant increases in the tissue-specific activities of the brush-border enzymes (15, 17). After birth, rapid small intestine growth and maturation are maintained in response to enteral food intake (2, 16). The regulatory factors involved in small intestine growth in the perinatal period remain relatively poorly understood, although local and systemic agents are known to play a role.

The actions of GLP-2 are mediated via a specific receptor belonging to the glucagon-secretin G protein-coupled receptor superfamily (14). The GLP-2 receptor (GLP-2R) mRNA has recently been identified in the small intestine of fetal and postnatal rats (13). This report, together with our earlier evidence showing that GLP-2 stimulates small intestine growth in the premature neonatal pig (3), suggests that the small intestine is responsive to GLP-2 early in development. Despite the evidence that proglucagon and GLP-2R mRNA are expressed in the fetus, there is no evidence as to whether the biologically active form of GLP-2 is present in the fetal circulation or whether, indeed, the fetal intestine is responsive to GLP-2. In the present study, we sought to determine whether the fetal intestine was responsive to exogenous GLP-2. Having established that premature newborn piglets are responsive to GLP-2 (3), we compared the effects of GLP-2 in fetuses with those in TPN-fed piglets delivered at full

Address for reprint requests and other correspondence: Y. M. Petersen, Div. of Animal Nutrition, Royal Veterinary and Agricultural University, 3 Grønnegårdsvej, DK-1870 Frederiksberg, Copenhagen, Denmark (E-mail: yms@kvl.dk).
term. The specific objectives of the study were to compare fetal and neonatal pigs with respect to 1) the sites of GLP-2R expression and the concentrations of GLP-2 in the circulation, 2) the effects of GLP-2 on gut morphology and crypt cell proliferation, and 3) the effects of GLP-2 on lactase-phloridzin hydrodase (LPH), maltase-glucoamylase (MGA), sucrase-isomaltase (SI), aminopeptidase N (ApN), aminopeptidase A (ApA), and DPP IV mRNA levels and enzyme activities.

METHODS

Fetal animals. Six pregnant sows (Large White × Landrace) of known gestational age (98 ± 2 days, full term = 115 ± 2 days) were sedated (azeparone, 0.05 ml/kg im; Janssen, Beerse, Belgium), and a superficial ear vein was catheterized under local anesthesia (lidocaine; Lignocaine, Willotox, Crawley, UK). General anesthesia was induced with thiopental sodium (5 mg/kg; Abbott Laboratories, North Chicago, IL) and isoflurane (1–2% in oxygen; Abbott Laboratories) using an endotracheal tube. The sow was covered with insulating blankets to prevent anesthesia-induced hypothermia, and great care was taken to maintain aseptic conditions around the ventral flank incision. A small incision (5 cm) was made in a relatively nonvascular area of the uterus, and the fetal allantoic and amniotic membranes were then carefully anchored onto the uterine wall incision. The head of a fetus was exteriorized and covered with a wet heating pad to prevent dehydration and cooling. A ventral midline incision was made in the fetal neck, and surrounding tissue was gently separated to expose the carotid artery. A polyvinyl catheter was inserted into the carotid artery and secured with sutures. The fetal skin, fetal membranes, and uterine incision were sutured separately. Antibiotics (50 mg ampicillin; Penbritin, Beecham Laboratories) were administered to each operated fetus by injection into the amniotic cavity. With the use of this procedure, two more fetuses were randomly selected and catheterized. The duration of surgery was 3–8 h. Antibiotics were given to the sow for 4 days (20 ml/day im; Streptocillin, Boeringer Ingelheim, Copenhagen, Denmark) after surgery, and progestosterone was administered (50 mg/days im) 3 days after surgery as a precaution against preterm labor. Preliminary observations showed that GLP-2 infusion into operated pig fetuses could pass into the maternal circulation. Therefore, to prevent possible cross-contamination of the control fetuses with GLP-2 via the placental circulation, all three catheterized fetuses from each sow were assigned to be vehicle-infused controls or GLP-2-infused fetes. Fetal well-being was assessed daily by blood gas analyses (pH, P02, P CO2) using a blood gas analyzer (NOVA Biomedical, Waltham, MA). Of the 18 operated fetuses, 5 died before completion of the study.

Full-term neonatal animals. A total of 14 cross-bred pigs (Large White × Landrace) were obtained by cesarean delivery (full term = 115 ± 2 days) from two different litters. Twenty hours before delivery, pregnant sows were injected with prostaglandin F2 (175 µg im, cloprostenol; Estrumate, Pitman-Moore, Harefield, UK) to initiate the normal metabolic and endocrine changes that occur in the fetus and sow before spontaneous delivery (20). The delivered piglets were rapidly removed from the uterus, and the umbilical cord was ligated and transected. Each piglet was fitted with a catheter into the dorsal aorta inserted via the transected umbilical artery (infant feeding tube 4-F; Portex).

The newborn pigs were housed individually in infant incubators (Air-Shields, Hatboro, PA) maintained at 32–34°C and 80–100% moisture and administered a continuous parenteral infusion of an elemental nutrient solution via the umbilical catheter. The parenteral diet consisted of free amino acids, glucose, lipid, minerals, and vitamins and has been described previously (3). Parenteral nutrition was administered continuously starting 6 h after delivery, and after an initial 2-day period in which the animals received 50% of the total intake, the animals received 730 KJ·kg−1·day−1 and 8 g amino acid·kg−1·day−1; fluid intake was 230 mL·kg−1·day−1 for the remainder of the experimental period. To provide some passive immunity, the piglets were given a total of 15 ml of maternal serum over the first 24 h via the arterial catheter. The serum was produced from maternal blood collected from a uterine vein at the time of surgery. The piglets were weight matched and assigned to control and GLP-2-treated groups. All procedures were approved by the National Committee on Animal Experimentation, Denmark.

Treatment protocol. Fetal and neonatal pigs received two daily 2-h intravenous infusions of human GLP-2 (12.5 nmol/kg; a generous gift from L. Thin, Novo Nordisk, Bagsværd, Denmark) or the vehicle (0.1% porcine serum albumin in 0.9% buffered saline) starting at 0800 and 1600 over a 5- to 6-day period. A 2-ml arterial blood sample was collected daily at 0800 before the GLP-2 and vehicle infusions to determine basal GLP-2 concentrations.

Tissue collection. After 6 days, the operated fetal pigs were delivered by cesarean section as described above. A corresponding number of fetal pigs, which had not been operated on previously, were also delivered from the same litter and designated unoperated fetal pigs. The unoperated fetal pigs were used to enable a statistical adjustment of results (e.g., organ weights and enzyme activities) for possible sow (litter) effects. Fetal and neonatal pigs were immediately euthanized with an overdose of pentobarbitone sodium (200 mg/kg iv). The abdomen was opened, and the entire small intestine distal to the ligament of Treitz was removed and immediately flushed with ice-cold physiological saline. Stomach, small intestine, and colon were weighed. The small intestine was separated into three segments of equal length (proximal, middle, and distal), and samples from the middle of each section, together with samples of stomach, colon, and kidney, were individually snap-frozen in liquid nitrogen and stored at −70°C. Individual samples of proximal and distal small intestine were fixed in 4% buffered formaldehyde for 24 h (for morphometric analyses) or in Carnoy’s fluid (60% ethanol-30% glacial acetic acid-10% chloroform) for 8 h (for cell proliferation analyses). After fixation, the samples were stored in 70% ethanol at 4°C. The proportion of dry mucosa was determined after the mucosa and the muscularis layers were dried at 50°C for 72 h.

GLP-2 radioimmunoassay. Circulating GLP-2 levels were measured with an NH2-terminal-specific antiserum. This assay solely detects the NH2-terminal region of porcine GLP-2 and has thoroughly been described previously (8). The experimental limit of detection of this assay is 5 pM, and the intra-assay coefficient of variation is 5% at 40 pM.

Morphometry. Formaldehyde-fixed samples were embedded in paraffin, sectioned (5 µm), and stained with hematoxylin and eosin. Mean villus height and crypt depth were measured in 15 vertically well-oriented villus crypt columns, and muscle thickness was determined in 15 cross sections of small intestine and averaged by a blinded observer using an Axioshot microscope (Carl Zeiss) and NIH Image software (version 1.60, National Institutes of Health, Bethesda, MD).

In vivo bromodeoxyuridine labeling. In vivo crypt cell proliferation was measured using 5-bromodeoxyuridine (BrdU) as previously described (3). Briefly, fetuses (in utero) and
neonatal pigs were infused with an intravenous bolus of 
BrdU (50 mg/kg; Sigma Aldrich) 4 h before they were killed. 
Sections (5 μm) fixed in Carnoy’s fluid were incubated with a 
mouse anti-BrdU/nuclease (Amersham Pharmacia Biotech, 
Piscataway, NJ) and then with biotinylated universal second-
ary antibody (anti-mouse IgG2a; The Binding Site). BrdU-labeled 
cells were visualized using 3,3′-diaminobenzidine substrate kit for 
peroxidase (Vector Laboratories, Burlingame, CA), and then slides 
were counterstained with 0.1% hematoxylin. The number of nuclei 
stained brown by BrdU in 10 vertically well-oriented crypts per hour of 
BrdU infusion was determined and expressed as a percentage of the total 
number of nuclei per crypt.

**RNA extraction.** Total RNA was extracted from individu-
ally frozen samples of fundus, proximal and distal small 
intestine, colon, and kidney collected from each pig (4). The 
RNA was then dissolved in 0.1% diethylpyrocarbonate 
(DEPC)-treated water and recovered (absorbance at 260 nm) 
and purity (ratio of absorbance at 260 nm to absorbance at 
280 nm) were determined with an ultraviolet spectrophotom-
eter (GeneQuant pro, Amersham Pharmacia Biotech, Cam-
bridge, UK).

**Reverse transcription-polymerase chain reaction.** Individ-
ual samples of total RNA (3 μg), random decamer primers (50 
μM; Ambion, Austin, TX), dNTP mix (10 mM each dGTP, 
dATP, dTTP, and dCTP; Life Technologies), and DEPC-
M; Ambion, Austin, TX), dNTP mix (10 mM each dGTP, 
m/MgCl 2 (Life Technologies), autoclaved distilled water and 1 U of Taq DNA 
polymerase (Life Technologies), 1 μl of 18S rRNA internal 
standards (Ambion, Austin, TX), and specific sense and antisense oligonucleotides at 0.5 μM each (Table 1; TAG, 
Copenhagen, Denmark). The samples were denatured in a 
thermal cycler (model PTC-100, MJ Research, Cambridge, 
MA) at 95°C for 2 min and underwent amplification cycles 
with denaturation at 95°C for 1 min, annealing for 30 s at 
51–59°C, and extension for 1 min at 72°C. An additional 
extension at 72°C for 10 min was performed, and the samples 
were then cooled to 4°C. To control for the absence of genomic 
DNA contamination, PCRs were carried out on samples in 
which the reverse transcription enzyme had been excluded in 
the reverse transcription reaction. Amplification products 
were electrophoresed on a 1% agarose gel (BDH Laboratory, 
Poole, UK) in Tris-borate-EDTA buffer and visualized by 
staining with 0.15% ethidium bromide.

To compare enzyme mRNA levels after GLP-2 treatment, 
we first determined the optimal annealing temperature for 
each primer set (Table 1) and then the range of PCR cycles 
over which the amplification efficiency of the reaction was at 
its maximum (for the specific primer set and the 18S rRNA 
standards) and remained constant using a pooled sample of 
proximal or distal cDNA from fetuses or neonates. A number 
of cycles within the linear range of amplification of each 
primer set were selected, and the PCRs were performed on 
individual samples for comparison. The relative abundance 
of the enzyme PCR products on the gels was quantified by an 
optical densitometry reading (Image Pro Plus 4.1 software) of 
PCR bands on digitalized pictures (BioCapt 97 software, 
Vilber Lourmat, Cedex, France) of gels. For each of the two 
age groups (fetuses and neonates), individual enzyme mRNA 

<table>
<thead>
<tr>
<th>Oligonucleotides</th>
<th>Annealing Temperatures and Cycles</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLP-2 VR</td>
<td>34°C, 31 cycles</td>
<td>5'-ACCTTGCACGTGATGTAACAC-3'</td>
</tr>
<tr>
<td>Sense</td>
<td></td>
<td>5'-GTGTTCTCCAGGTGTGCACG-3'</td>
</tr>
<tr>
<td>Antisense</td>
<td></td>
<td>5'-CTCAGGGTAAAGTGTTGAC-3'</td>
</tr>
<tr>
<td>LPH</td>
<td>51°C, 32 and 29 cycles</td>
<td>5'-AGCATGAAATGCAAAGGAAGAAG-3'</td>
</tr>
<tr>
<td>Sense</td>
<td></td>
<td>5'-ACGAAGTGGCCAGAAGCTTG-3'</td>
</tr>
<tr>
<td>Antisense</td>
<td></td>
<td>5'-AGAAGCTTGGTGACATCC-3'</td>
</tr>
<tr>
<td>MGA</td>
<td>56°C, 32 and 27 cycles</td>
<td>5'-ACCTTTGACGTGATGTAACAC-3'</td>
</tr>
<tr>
<td>Sense</td>
<td></td>
<td>5'-GTGTTCTCCAGGTGTGCACG-3'</td>
</tr>
<tr>
<td>Antisense</td>
<td></td>
<td>5'-CTCAGGGTAAAGTGTTGAC-3'</td>
</tr>
<tr>
<td>SI</td>
<td>56°C, 32 and 29 cycles</td>
<td>5'-ACGAAGTGGCCAGAAGCTTG-3'</td>
</tr>
<tr>
<td>Sense</td>
<td></td>
<td>5'-AGAAGCTTGGTGACATCC-3'</td>
</tr>
<tr>
<td>Antisense</td>
<td></td>
<td>5'-ACGAAGTGGCCAGAAGCTTG-3'</td>
</tr>
<tr>
<td>ApN</td>
<td>58°C, 29 and 27 cycles</td>
<td>5'-ACATCAGCTCAGCCACTC-3'</td>
</tr>
<tr>
<td>Sense</td>
<td></td>
<td>5'-ACATCAGCTCAGCCACTC-3'</td>
</tr>
<tr>
<td>Antisense</td>
<td></td>
<td>5'-ACGTCTTACCATCCACCT-3'</td>
</tr>
<tr>
<td>ApA</td>
<td>56°C, 29 and 26 cycles</td>
<td>5'-ACATCAGCTCAGCCACTC-3'</td>
</tr>
<tr>
<td>Sense</td>
<td></td>
<td>5'-ACATCAGCTCAGCCACTC-3'</td>
</tr>
<tr>
<td>Antisense</td>
<td></td>
<td>5'-ACATCAGCTCAGCCACTC-3'</td>
</tr>
<tr>
<td>DPP IV</td>
<td>59°C, 28 and 27 cycles</td>
<td>5'-CCTCCGGCGTCAGCAGCAG-3'</td>
</tr>
<tr>
<td>Sense</td>
<td></td>
<td>5'-CCTCCGGCGTCAGCAGCAG-3'</td>
</tr>
<tr>
<td>Antisense</td>
<td></td>
<td>5'-CCTCCGGCGTCAGCAGCAG-3'</td>
</tr>
</tbody>
</table>

GLP-2R glucagon-like peptide-2 receptor; LPH, lactase-phloridzin hydrolase; MGA, maltase-glucoamylase; SI, sucrase-isomaltase; ApN and ApA, aminopeptidase N and A; DPP IV, dipeptidylpeptidase IV.

---

**Table 1. Oligonucleotide sense and antisense sequences, annealing temperatures, and cycle number in fetuses and neonates, respectively**

---

**GLP-2 effects in the fetus and neonate**

PCR amplification of GLP-2R cDNA (fundus, proximal and 
distal small intestine, colon, and kidney) and brush-border 
enzyme cDNA (proximal and distal small intestine) 
was performed on individual samples. Three microliters of 
the cDNA from the reverse transcription reaction were added to 
17 μl of a PCR master mix consisting of 10× PCR buffer 
minus Mg [200 mM Tris- HCl (pH 8.4), 500 mM KCl; Life 
Technologies], 10 mM dNTP mix, 50 mM MgCl 2 (Life 
Technologies), autoclaved distilled water and 1 U of Taq DNA 
polymerase (Life Technologies), 1 μl of 18S rRNA internal 
standards (Ambion, Austin, TX), and specific sense and antisense oligonucleotides at 0.5 μM each (Table 1; TAG, 
Copenhagen, Denmark). The samples were denatured in a 
thermal cycler (model PTC-100, MJ Research, Cambridge, 
MA) at 95°C for 2 min and underwent amplification cycles 
with denaturation at 95°C for 1 min, annealing for 30 s at 
51–59°C, and extension for 1 min at 72°C. An additional 
extension at 72°C for 10 min was performed, and the samples 
were then cooled to 4°C. To control for the absence of genomic 
DNA contamination, PCRs were carried out on samples in 
in which the reverse transcription enzyme had been excluded in 
the reverse transcription reaction. Amplification products 
were electrophoresed on a 1% agarose gel (BDH Laboratory, 
Poole, UK) in Tris-borate-EDTA buffer and visualized by 
staining with 0.15% ethidium bromide.

To compare enzyme mRNA levels after GLP-2 treatment, 
we first determined the optimal annealing temperature for 
each primer set (Table 1) and then the range of PCR cycles 
over which the amplification efficiency of the reaction was at 
its maximum (for the specific primer set and the 18S rRNA 
standards) and remained constant using a pooled sample of 
proximal or distal cDNA from fetuses or neonates. A number 
of cycles within the linear range of amplification of each 
primer set were selected, and the PCRs were performed on 
individual samples for comparison. The relative abundance 
of the enzyme PCR products on the gels was quantified by an 
optical densitometry reading (Image Pro Plus 4.1 software) of 
PCR bands on digitalized pictures (BioCapt 97 software, 
Vilber Lourmat, Cedex, France) of gels. For each of the two 
age groups (fetuses and neonates), individual enzyme mRNA
was run on the same gel for all the pigs. The density of each enzyme cDNA band was expressed relative to the density of its corresponding 18S rRNA band. To confirm the identity of each PCR product, the cDNA was extracted from the gel (QIAquick gel extraction kit, West Sussex, UK), sequenced (TAG, Copenhagen, Denmark), and entered into BLAST (National Center for Biotechnology Information).

**Primers.** Oligonucleotides to recognize the pig GLP-2R were designed from regions of high homology between the GLP-2R cDNA sequences in rat (accession number AF105368) and human (AF105367). The sense and antisense oligonucleotides corresponded to nucleotides 529–548 and 964–983, respectively, in rat and human. Oligonucleotides were designed to identify MGA (491 bp; NM_004668), ApN (459 bp; Z29522), ApA (554 bp; U66371), and DPP IV (587 bp; X73277). Oligonucleotides to recognize pig LPH were designed from regions of high homology among LPH cDNA sequences from human (NM_002299), rat (X56747), and rabbit (Z27167) LPH. Oligonucleotides to recognize pig SI were designed from regions of high homology between SI cDNA sequences from human (NM_001041) and rat (NM_013061). The oligonucleotides recognized a 540- and a 484-bp product for LPH and SI, respectively. In the neonate pig gut, disaccharidase activities tend to be highest in the proximal intestine (2.4 ± 0.6 vs. 2.0 ± 0.2) and were unchanged in the fundus (0.72 ± 0.13 vs. 0.67 ± 0.04), distal intestine (2.4 ± 0.6 vs. 1.6 ± 0.6), and colon (3.7 ± 0.6 vs. 3.1 ± 0.3). GLP-2 mRNA was not present at detectable levels in fetal and neonatal kidney (Fig. 1).

**Body weights and gut growth.** In fetuses, body weights at delivery did not differ among the groups of unoperated, GLP-2-infused, and vehicle-infused control animals (1,193 ± 35, 1,168 ± 53, and 1,207 ± 46 g, respectively). Fetal GLP-2 infusion had no effect on small intestine weight (Fig. 2), proportion of dry mucosa (73 ± 1 vs. 72 ± 2%), and colon weight (Fig. 2). Similarly, GLP-2 had no effect on small intestine villus height (Fig. 3A), crypt depth or muscularis thickness, and rates of cell proliferation (Fig. 3B). Cell proliferation rates were, however, significantly higher in the proximal than in the distal intestine (P < 0.05).

In neonates, body weights and daily weight gain were not different between those treated with GLP-2 (GLP-2 and vehicle infusion), sow, intestinal region (proximal, middle, and distal), and treatment × region as fixed effects (18). For the fetuses, values from unoperated pigs were included in the model to allow correction for sow (litter effects). The results are expressed as the adjusted least-squares means ± SE or means ± SE, and differences between two means were tested by the least significant difference test or Student’s t-test. P = 0.05 was used as the critical level of significance for all statistical evaluations.

**RESULTS**

**Circulating GLP-2 and tissue GLP-2R.** At 98 ± 2 days of gestation (the time of fetal surgery), mean plasma GLP-2 levels were below the limit of detection (<5 pM), but the values increased significantly in the prenatal period to 11 ± 1 (SE) pM at full term (P < 0.05). Across the days of the experiment (GLP-2 or vehicle infusions), the basal GLP-2 levels were significantly higher in both groups of GLP-2-infused pigs (252 ± 98 and 309 ± 120 pM in fetuses and neonates, respectively) than in their corresponding controls (14 ± 2 and 23 ± 5 pM, respectively).

The partial pig GLP-2R cDNA sequenced showed 86% and 84% similarity to human and rat GLP-2R. The GLP-2R transcript was expressed in fetal and neonatal pigs (Fig. 1). The density ratio of GLP-2R mRNA to 18S rRNA was significantly increased in the proximal small intestine of neonates compared with fetuses (5.0 ± 0.6 vs. 2.0 ± 0.2) and was unchanged in the fundus (0.72 ± 0.13 vs. 0.67 ± 0.04), distal intestine (2.4 ± 0.6 vs. 1.6 ± 0.6), and colon (3.7 ± 0.6 vs. 3.1 ± 0.3). GLP-2R mRNA was not present at detectable levels in fetal and neonatal kidney (Fig. 1).

![Image](http://ajpregu.physiology.org/)
and controls (1,651 ± 38 vs. 1,641 ± 30 g and 52 ± 5 vs. 44 ± 2 g/day, respectively). GLP-2 significantly increased small intestine weight (Fig. 2), proportion of dry mucosa (70 ± 1 vs. 63 ± 1%), and colon weight (Fig. 2). In the proximal and distal small intestine, GLP-2 infusion significantly increased villi height (Fig. 3A), with no effects on crypt depth and muscle thickness. GLP-2 had no effect on the rates of crypt cell proliferation (Fig. 3B), and the rates did not differ between the proximal and distal intestine.

Disaccharidase and peptidase mRNA levels and activities. In fetuses, GLP-2 significantly increased ApN mRNA levels and activity in the distal small intestine but had no effect on other enzymes in the proximal intestine (LPH, MGA, and SI) and distal intestine (ApA and DPP IV; Figs. 4–6). In neonatal pigs, GLP-2 significantly increased MGA mRNA levels and maltase activity in the proximal small intestine but had no effect on other enzymes in the proximal intestine (LPH and SI) and distal intestine (ApN, ApA, and DPP IV; Figs. 4–6).

**DISCUSSION**

The main goal of this study was to determine whether the fetal intestine is responsive to exogenous GLP-2. Given evidence that GLP-2 is a potent gut trophic factor, our end points were measures of intestinal growth and function. We have shown that the GLP-2R is expressed in the gastrointestinal tract of pig fetuses and neonates, consistent with earlier studies in adult rats (14, 24). In newborn pigs, exogenous GLP-2 induced a marked increase in intestinal mucosal mass and villus height. Because the pigs were maintained on TPN, this effect occurred in the absence of luminal...
nutrient stimuli. The trophic action of GLP-2 may be mediated via an inhibition of mucosal proteolysis and apoptosis, as shown in our previous study on premature TPN-fed pigs (3). GLP-2 had no effect on cell proliferation in premature pigs (3) or in fetal and full-term newborn pigs (present study), even though an increase in cell proliferation has previously been reported in studies on adult rodents (6). It has been uncertain whether the trophic actions of GLP-2 are mediated directly via receptors expressed on enterocytes. Recent studies identifying the receptor protein solely on the enteroendocrine cells have led to the speculation that the trophic effects of GLP-2 are probably mediated indirectly via peptidergic mediators (24). Thus our results suggest that some component of this putative paracrine receptor signaling mechanism is absent or not fully developed in the period around birth.

Endogenous GLP-2 secretion from intestinal L cells is primarily regulated by enteral nutrient intake (2). Circulating GLP-2 is undetectable in the fetus before birth, suggesting that the luminal contents (amnion and meconium) to which the fetus is exposed in utero do not play a major role in regulating GLP-2 secretion. In contrast to the effects of GLP-2 in neonates, we show that GLP-2 infusion had no effect on mucosal growth and villus height in the fetuses. The absence of a trophic effect of GLP-2 in the fetal gut raises several possibilities. First, the fetal GLP-2R may not be functionally coupled to the secondary pathways (e.g., cAMP, phosphatidylinositol 3-kinase, and mitogen-activated protein kinases) that are known to mediate changes in epithelial cell proliferation (9, 14). Second, it is possible that the fetal GLP-2R is indeed functional but that the enteral stimulus of swallowed amniotic fluid is sufficient to render the intestinal mucosa in-

Fig. 5. A: MGA mRNA levels in fetal and neonatal pigs treated with GLP-2 and in controls (n = 6–7). B: ApN mRNA levels in fetal and neonatal pigs treated with GLP-2 and in controls (n = 6–7). 18S rRNA internal standards were included in the PCR.

Fig. 6. Brush-border enzyme activities in proximal (LPH, MGA, and SI) and distal (ApN, ApA, and DPP IV) small intestine of fetal (A) and full-term newborn pigs administered GLP-2 (B) and in controls. See Fig. 2 legend for explanation of bars. *Significant differences between each age group (P < 0.05).
sensitive to the trophic effects of pharmacological plasma GLP-2 concentrations. However, this still implies that the fetal intestine is less responsive to GLP-2, because previous studies in rodents have shown that exogenous GLP-2 treatment markedly stimulates intestine growth, even when animals are fed enterally (6, 12).

A secondary end point we used to assess GLP-2 responsiveness was intestinal digestive function, based on the expression and activity of several intestinal disaccharidases and peptidases. There are few studies in the literature focusing on the effect of GLP-2 on the expression and activity of brush-border enzymes. GLP-2 increases SI expression in the rat ileum (11) but has no effect on maltase, SI, LPH, and DPP IV activities in mouse duodenum (1). In the present study, disaccharidase and peptidase expression have been measured in the proximal and distal small intestine, respectively, to provide a more complete overview of the effects of GLP-2 on enzyme regulation in the small intestine. In the fetuses, GLP-2 administration selectively increased ApN mRNA levels and activity, whereas in the neonates, GLP-2 had a significant stimulatory effect only on maltase mRNA levels and activity. The increase in ApN and maltase mRNA levels, together with increased activity, suggests that GLP-2 regulates the function of these enzymes at the level of transcription and that the intestine of fetal and neonatal pigs is responsive to GLP-2 treatment. The effects of GLP-2 on the fetal intestine did not, however, reflect the changes in enzyme activities normally occurring in the perinatal period. Therefore, our findings suggest that GLP-2 has a limited role in the normal maturation of small intestine growth and function at this time. Furthermore, the changes in intestine growth and function in GLP-2-infused fetal and neonatal piglets do not necessarily indicate that GLP-2 is involved in the normal regulation of gut physiology, inasmuch as pharmacological concentrations of circulating GLP-2 were present in the circulation of these animals.

We were unable to detect the active GLP-2 in plasma from 98-day fetuses (<5 pM), but there were measurable amounts in the 105-day fetus, and the levels increased further into the postnatal period of TPN-fed neonates (23 ± 5 pM). A recent study in rodents suggests that GLP-2 is present in tissue extracts and media derived from cultured fetal intestinal tissue (13). Moreover, the GLP-2 concentration in intestinal tissue increased during early postnatal development, whereas that in plasma decreased. Although we have not measured the peptide concentrations in intestinal tissue, using this NH2-terminal assay, we have measured GLP-2 in pig plasma from a range of ages, including the earliest detectable fetal age (i.e., 105 days of gestation) in the present study and in young pigs at 14–30 days of age (2, 3, 22). Across this age range, the basal circulating GLP-2 concentration in pigs that were fasted or fed exclusively via the parenteral route ranged from 14 pM in fetuses to as high as 30 pM at 30 days of age. Similarly, the GLP-2 concentrations in pigs that were enterally fed sow’s milk or a cow’s milk formula ranged from ~50 pM in premature newborn pigs to as high as 100 pM in 30-day-old pigs. Taken together, these observations suggest that, in the pig at least, the circulating concentration, and perhaps secretion, of GLP-2 increases during early postnatal development. Changes in the secretion of GLP-2 can be due to a number of factors, such as the presence of the proconvertase enzymes involved in the specific processing of proglucagon to yield GLP-2; however, the ontogeny of the proconvertase enzymes has not been studied in the pig. Although it is certain that enteral nutrition is a major stimulus for intestinal GLP-2 secretion, it will be of interest to establish whether it results from merely an increase in the basal concentration or whether intestinal GLP-2 secretion becomes more responsive to enteral nutrition with advancing age.

In conclusion, we have shown that the GLP-2R mRNA is expressed in the gastrointestinal tissues of the fetal and neonatal pig and that the signaling pathways involved in the trophic responses of the small intestine to GLP-2 are stimulated by birth and/or gut maturation. In addition, the stimulatory effect of exogenous GLP-2 on maltase and ApN function in fetal and neonatal pigs suggests that the stage of small intestine development affects the responsiveness to GLP-2.

**Perspectives**

A better understanding of the factors involved in regulating small intestine growth is crucial in the design of treatments aimed at improving small intestine function. In this study, we have shown that the small intestine was responsive to GLP-2 and that the extent of this responsiveness was associated with gut maturity. This early responsiveness of the small intestine suggests that GLP-2 could potentially be used to enhance small intestine growth or function in neonates maintained on TPN. Before such clinical trials can be performed, however, further studies are needed to determine the developmental profile (localization and distribution) of the GLP-2R protein and the mechanisms (direct or indirect) by which the actions of GLP-2 are mediated.

We gratefully acknowledge the contribution of Bollette Hartmann and Jens J. Holst in the running of the GLP-2 radioimmunoassays. We thank Mette Schmidt, Jan Elnif, and Randy Buddington for assistance and valuable discussions and Ebba de Neergaard Harri-son, Anna Siekierska, Inger Heintze, Bente Synnesvedt, Ann Pedersen, and Xiaoyan Chang for skilled technical assistance. Susanne Gerlac and the Department of Pharmacy at Righospitalet (Copenha-gen) are gratefully acknowledged for help in the preparation of the TPN solutions.

This work was supported by Danish Agricultural and Veterinary Research Council Program 9702803, a Ph.D. student scholarship from the Danish Royal Veterinary and Agricultural University, and a travel grant from Nortoft Thomsen Legat.

**REFERENCES**

1. Brubaker PL, Izzo A, Hill M, and Drucker DJ. Intestinal function in mice with small bowel growth induced by glucagon-
GLP-2 EFFECTS IN THE FETUS AND NEONATE


